REMARKS

Claims 4-17 are pending.

Objections

The Examiner asserts that the title of the invention is not descriptive. Applicants have amended the title of the application to address the Examiner's concerns.

Utility

Claims 4-17 were rejected on the assertion that the claimed invention lacks utility. The Examiner asserts that the data set forth in the specification are preliminary at best because the specification does not teach the expression of the PRO1864 polypeptide nor any particular biological activity of the polypeptide. According to the Examiner, the specification provides no information regarding PRO1864 polypeptide levels in tumor samples relative to normal samples nor is there any information as to the significance of the expression.

The Examiner asserts that Mr. Grimaldi's first Declaration is unpersuasive. Hu is cited as teaching that, for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a known role in the disease. LaBaer is cited as teaching that reports of mRNA or protein changes of as little as two-fold are not uncommon, and that, although changes of this magnitude may turn out to be important, most are attributable to disease-independent differences between the samples.

The Examiner asserts that Mr. Grimaldi's second Declaration and the Declaration by Dr. Polakis are unpersuasive. The Examiner asserts that the central dogma established by Crick only speaks to the flow of information but does not draw quantitative correlations between levels of mRNA and protein. The Examiner asserts that the Alberts, Lewin and Meric references submitted in support of the Declarations support the fact that further research would have to be carried out to determine if the polypeptide expression levels track with the expression levels of the corresponding mRNA.

The Examiner also reiterates his position that Gökman-Polar and Gygi teach that there is no necessary correlation between mRNA levels and protein levels and that one cannot predict protein levels from mRNA.

Greenbaum et al. is cited as cautioning against assuming that mRNA levels are generally correlative of protein levels. The Examiner reiterates his previous rejection asserting that Haynes et al. teach that there is no strong correlation between polypeptide and mRNA levels. Lian et al. is cited as showing a similar lack of correlation in mammalian cells. Fessler et al. is also cited as finding a "[p]oor concordance between mRNA transcript and protein expression changes" in human cells. Hanash [a] (Nature Reviews, Applied Proteomics Collection, pp. 9-14, March 2005) is cited as teaching that "There is a need to profile gene expression at the level of the proteome and to correlate changes in gene-expression profiles with changes in proteomic profiles. The two are not always linked-numerous alterations occur in protein levels that are not reflected at the RNA level." Hanash et al. [b] (The Pharmacogenomics Journal, 3(6):308-311, 2003, IDS reference 9 filed 9/16/2005) is cited as teaching that "However perfected DNA microarrays and their analytical tools become for disease profiling, they will not eliminate a pressing need for other types of profiling technologies that go beyond measuring RNA levels, particularly for disease-related investigations." (see page 311).

According to the Examiner, the literature teaches that RNA expression cannot inevitably be correlated with levels of the encoded polypeptide and one skilled in the art would not presume that the levels of RNA are necessarily predictive of the levels of the encoded polypeptide given the distinct regulation of transcription and translation as evidenced by Hu et al., LaBaer, Hanna et al., Alberts[a], Alberts[b], Lewin, Zhigang et al., Meric et al., Gökman-Polar, Gygi et al., Greenbaum et al., Haynes et al., Lian et al., Fessler et al., Hanash S. [a] and Hanash et al. [b].

Utility – Legal Standard

As previously noted, according to the Utility Examination Guidelines ("Utility Guidelines"), 66 Fed. Reg. 1092 (2001) an invention complies with the utility requirement of 35 U.S.C. § 101, if it has at least one asserted "specific, substantial, and credible utility" or a "well-established utility."

Under the Utility Guidelines, a utility is "specific" when it is particular to the subject matter claimed. For example, it is generally not enough to state that a nucleic acid is useful as a diagnostic tool without also identifying the condition that is to be diagnosed.

The requirement of "substantial utility" defines a "real world" use, and derives from the Supreme Court's holding in *Brenner v. Manson*, 383 U.S. 519, 534 (1966) stating that "The basic *quid pro quo* contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility." In explaining the "substantial utility" standard, M.P.E.P. § 2107.01 cautions, however, that Office personnel must be careful not to interpret the phrase "immediate benefit to the public" or similar formulations used in certain court decisions to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. "Rather, *any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient,* at least with regard to defining a 'substantial' utility." (M.P.E.P. § 2107.01, emphasis added).

The mere consideration that further experimentation might be performed to more fully develop the claimed subject matter does not support a finding of lack of utility. M.P.E.P. § 2107.01 III cites *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995) in stating that "Usefulness in patent law ... necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans." Further, "to violate § 101 the claimed device must be totally incapable of achieving a useful result." *Juicy Whip Inc. v. Orange Bang Inc.*, 51 U.S.P.Q.2d 1700 (Fed. Cir. 1999), *citing Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555, 1571 (Fed. Cir. 1992).

Indeed, the Guidelines for Examination of Applications for Compliance With the Utility Requirement, set forth in M.P.E.P. § 2107 II(B)(1) gives the following instruction to patent examiners: "If the applicant has asserted that the claimed invention is useful for any particular practical purpose ... and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility."

Finally, in assessing the credibility of the asserted utility, the M.P.E.P. states that "to overcome the presumption of truth that an assertion of utility by the applicant enjoys" the PTO must establish that it is "more likely than not that one of ordinary skill in the art would doubt (i.e., 'question') the truth of the statement of utility." M.P.E.P. § 2107.02 III A. The M.P.E.P. cautions that:

> Rejections under 35 U.S.C. 101 have been **rarely sustained** by federal courts. Generally speaking, **in these rare cases**, the 35 U.S.C. 101 rejection was sustained [] because the **applicant ... asserted a utility that could only be true if it violated a scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art**. M.P.E.P. § 2107.02 III B., citing *In re Gazave*, 379 F.2d 973, 978, 154 U.S.P.Q. 92, 96 (CCPA 1967) (underline emphasis in original, bold emphasis added).

Utility need NOT be Proved to a Statistical Certainty – a Reasonable Correlation between the Evidence and the Asserted Utility is Sufficient

As previously noted, an Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297 (CCPA 1974). *See, also In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *In re Irons*, 340 F.2d 974, 144 USPQ 351 (1965); *In re Sichert*, 566 F.2d 1154, 1159, 196 USPQ 209, 212-13 (CCPA 1977). Compliance with 35 U.S.C. § 101 is a question of fact. *Raytheon v. Roper*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983) cert. denied, 469 US 835 (1984). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the evidence, or "more likely than not" standard. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). This is stated explicitly in the M.P.E.P.:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. M.P.E.P. at § 2107.02, part VII (2004) (underline emphasis in original, bold emphasis added, internal citations omitted).

The PTO has the initial burden to offer evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). Only then does the burden shift to the Applicant to provide rebuttal evidence. *Id.* As stated in the M.P.E.P., such rebuttal evidence does not need to absolutely prove that the asserted utility is real. Rather, the evidence only needs to be reasonably indicative of the asserted utility.

In *Fujikawa v. Wattanasin*, 93 F.3d 1559, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996), the Court of Appeals for the Federal Circuit upheld a PTO decision that *in vitro* testing of a novel pharmaceutical compound was sufficient to establish practical utility, stating the following rule:

> [T]esting is often required to establish practical utility. But the test results **need not absolutely prove** that the compound is pharmacologically active. All that is required is that the tests be "*reasonably* indicative of the desired [pharmacological] response." In other words, there must be **a sufficient correlation** between the tests and an asserted pharmacological activity so as to convince those skilled in the art, **to a reasonable probability**, that the novel compound will exhibit the asserted pharmacological behavior." *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1564, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996) (internal citations omitted, bold emphasis added, italics in original).

While the *Fujikawa* case was in the context of utility for pharmaceutical compounds, the principals stated by the Court are applicable in the instant case where the asserted utility is for a therapeutic and diagnostic use – utility does not have to be established to an absolute certainty, rather, the evidence must convince a person of skill in the art "to a reasonable probability." In addition, the evidence need not be direct, so long as there is a "sufficient correlation" between the tests performed and the asserted utility.

The Court in *Fujikawa* relied in part on its decision in *Cross v. Iizuka*, 753 F.2d 1040, 224 U.S.P.Q. 739 (Fed. Cir. 1985). In *Cross*, the Appellant argued that basic *in vitro* tests conducted in cellular fractions did not establish a practical utility for the claimed compounds. Appellant argued that more sophisticated *in vitro* tests using intact cells, or *in vivo* tests, were necessary to establish a practical utility. The Court in *Cross* rejected this argument, instead favoring the argument of the Appellee:

> [*I*]*n vitro* results...are generally predictive of *in vivo* test results, i.e., there is a **reasonable correlation** therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are. [Appellee] has not urged, and rightly so, that there is an invariable exact correlation between *in vitro* test results and *in vivo* test results. Rather, [Appellee's] position is that successful *in vitro* testing for a particular pharmacological activity establishes a **significant probability** that *in vivo* testing for this particular pharmacological activity will be successful. *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985) (emphasis added).

The *Cross* case is very similar to the present case. Like *in vitro* testing in the pharmaceutical industry, those of skill in the field of biotechnology rely on the reasonable

correlation that exists between gene expression and protein expression (see below). Were there no reasonable correlation between the two, the techniques that measure gene levels such as microarray analysis, differential display, and quantitative PCR would not be so widely used by those in the art. As in *Cross*, Applicants here do not argue that there is "an invariable exact correlation" between gene expression and protein expression. Instead, Applicants' position detailed below is that a measured change in gene expression in cancer cells establishes a "significant probability" that the expression of the encoded polypeptide in cancer will also be changed based on "a reasonable correlation therebetween."

Taken together, the legal standard for demonstrating utility is a relatively low hurdle. An Applicant need only provide evidence such that it is **more likely than not that a person of skill in the art would be convinced, to a reasonable probability, that the asserted utility is true.** The evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. The Applicant **does not need to provide evidence such that it establishes an asserted utility as a matter of statistical certainty.**

Even assuming that the PTO has met its initial burden to offer evidence that one of ordinary skill in the art would reasonably doubt the truth of the asserted utility, Applicants assert that they have met their burden of providing rebuttal evidence such that it is more likely than not those skilled in the art, to a reasonable probability, would believe that the claimed invention is useful as a diagnostic tool for cancer.

Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

In an attempt to clarify Applicants' argument, Applicants offer a summary of their argument and the disputed issues involved. Applicants assert that the claimed polypeptides have utility as diagnostic tools for cancer, particularly melanoma. Applicants' asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that mRNA for the PRO1864 polypeptide is expressed at least two-fold higher in melanoma compared to normal skin tissue;

2. Applicants assert that it is well-established in the art that a **change** in the level of mRNA for a particular protein, e.g. an increase, generally leads to a corresponding **change** in the level of the encoded protein, e.g. an increase;

3. Given Applicants' evidence that the level of mRNA for the PRO1864 polypeptide is increased in melanoma compared to normal skin tissue, it is likely that the PRO1864 polypeptide is more highly expressed in melanoma compared to normal skin tissue;

4. Proteins which are differentially expressed in certain tumors are useful as diagnostic tools.

Applicants understand the PTO to be making several arguments in response to Applicants' asserted utility:

1. The PTO has challenged the reliability of the evidence reported in Example 18 and states that the specification does not teach the expression of the PRO1864 protein, any particular biological activity of the polypeptide, or the levels of the PRO1864 polypeptide in tumor samples relative to normal samples;

2. The PTO cites Hu *et al* and LaBaer *et al.* in support of its position that the differential mRNA expression data in Example 18 is insufficient to convey utility to the claimed polypeptides;

3. The PTO cites Crick, Gökman-Polar, Gygi, Greenbaum, Haynes , Lian et al., Fessler et al., Hanash [a], and Hanash [b] in support of its position that there is no correlation between mRNA levels and polypeptide levels. The PTO further maintains that Molecular Biology of the Cell, Lewin, Zhigang, and Meric support this position.

As detailed below, Applicants submit that the PTO has failed to demonstrate that this is one of the "rare cases" where the applicants have "asserted a utility that could only be true if it violated a scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art." M.P.E.P. § 2107.02 III B. First, the PTO has failed to offer any evidence to support its rejection of the data in Example 18 and the Declaration of Chris Grimaldi in support of these data. Second, Applicants submit that the cited references are not contrary to Applicants' arguments, and therefore are not evidence to support the PTO's position. Finally, even if the PTO has met its initial burden, Applicants have submitted enough rebuttal evidence such that it is **more likely than not** that a person of skill in

the art would be convinced, **to a reasonable probability,** that the asserted utility is true. As stated above, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute certainty.**

Applicants have established that the Gene Encoding the PRO1864 Polypeptide is Differentially Expressed in Certain Cancers compared to Normal Tissue

As previously stated, Applicants maintain that the gene expression data in the specification, Example 18, shows that the mRNA associated with protein PRO1864 was more highly expressed in melanoma compared to normal skin tissue. Gene expression was analyzed using standard semi-quantitative PCR amplification reactions of cDNA libraries isolated from different human tumor and normal human tissue samples. Identification of the differential expression of the PRO1864 polypeptide-encoding gene in tumor tissue compared to the corresponding normal tissue renders the molecule useful as a diagnostic tool for the determination of the presence or absence of tumor. In support, Applicants previously submitted as Exhibit 1 with the Amendment and Response to Office Action mailed April 29, 2005, a first Declaration of J. Christopher Grimaldi, an expert in the field of cancer biology. This Declaration explains the importance of the data in Example 18, and how differential gene and protein expression studies are used to differentiate between normal and tumor tissue (see Declaration, paragraph 7).

In paragraph 5 of his Declaration, Mr. Grimaldi states that the gene expression studies reported in Example 18 of the instant application were made from pooled samples of normal and of tumor tissues. Mr. Grimaldi explains that:

> The DNA libraries used in the gene expression studies were made from pooled samples of normal and of tumor tissues. *Data from pooled samples is more likely to be accurate than data obtained from a sample from a single individual.* That is, the detection of variations in gene expression is likely to represent a more generally relevant condition when pooled samples from normal tissues are compared with pooled samples from tumors in the same tissue type. (Paragraph 5) (emphasis added).

In paragraphs 6 and 7, Mr. Grimaldi explains that the semi-quantitative analysis employed to generate the data of Example 18 is sufficient to determine if a gene is over- or

under-expressed in tumor cells compared to corresponding normal tissue. He states that any visually detectable difference seen between two samples is indicative of at least a two-fold difference in cDNA between the tumor tissue and the counterpart normal tissue. Thus, the results of Example 18 reflect at least a two-fold difference between normal and tumor samples. He also states that the results of the gene expression studies indicate that the genes of interest "can be used to differentiate tumor from normal," thus establishing their reliability. He explains that, contrary to the PTO's assertions, "The precise levels of gene expression are irrelevant; what matters is that there is a relative difference in expression between normal tissue and tumor tissue." (Paragraph 7). Thus, since it is the relative level of expression between normal tissue and suspected cancerous tissue that is important, the precise level of expression in normal tissue is irrelevant. Likewise, there is no need for quantitative data to compare the level of expression in normal and tumor tissue. As Mr. Grimaldi states, "If a difference is detected, this indicates that the gene and its corresponding polypeptide and antibodies against the polypeptide are useful for diagnostic purposes, to screen samples to differentiate between normal and tumor."

The Examiner maintains that Mr. Grimaldi's Declaration is insufficient to overcome the utility rejection. According to the Examiner, the specification provides no information regarding PRO1864 polypeptide levels in tumor samples relative to normal samples and there is no information as to the significance of the expression. The Examiner asserts that, since the Declaration is limited to a discussion of data regarding the gene expression of the PRO1864 cDNA and not gene expression levels and polypeptide levels, it is insufficient to overcome the utility rejection. The Examiner further maintains that there is no evidentiary support to Dr. Grimaldi's statement that, if a difference in gene expression is detected, this indicates that the gene and its corresponding polypeptide and antibodies against the polypeptide are useful for diagnostic purposes.

As an initial matter, Applicants submit that the Declaration of Mr. Grimaldi is based on personal knowledge of the relevant facts at issue. Mr. Grimaldi is an expert in the field and conducted or supervised the experiments at issue. Applicants remind the PTO that "[o]ffice personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned." PTO Utility Examination Guidelines (2001) (emphasis added). In addition, declarations relating to issues of fact should not be summarily dismissed as

"opinions" without an adequate explanation of how the declaration fails to rebut the Examiner's position. *In re Alton* 76 F.3d 1168 (Fed. Cir. 1996). The PTO has not supplied any reasons or evidence to question the accuracy of the facts upon which Mr. Grimaldi based his statements. Mr. Grimaldi has personal knowledge of the relevant facts related to the data in Example 18, has based his statements on those facts, and the PTO has offered no reason or evidence to reject either the underlying facts or his statements. Therefore, the PTO should accept Mr. Grimaldi's statement that the genes of interest "can be used to differentiate tumor from normal."

Applicants maintain that they have shown that PRO1864 mRNA is differentially expressed and that therefore one would expect the PRO1864 polypeptide to also be differentially expressed. Because they are differentially expressed, the claimed polypeptides can be used as diagnostic agents for melanoma. Cancer diagnosis is both a substantial and a credible utility. In fact, the Revised Interim Utility Guidelines promulgated by the PTO recognize that proteins which are differentially expressed in cancer have utility. (*See* the caveat in Example 12 which state that the utility requirement is satisfied where a protein is expressed in melanoma cells but not on normal skin and antibodies against the protein can be used to diagnose cancer.) Furthermore, Applicants note that the PTO has issued several patents claiming differentially expressed polypeptides. (*See, e.g.,* U.S. Patent No. 6,414,117 and U.S. Patent No. 6,124,433, previously submitted as Exhibits 10 and 11 with the Amendment and Response to Office Action mailed April 29, 2005).

With respect to the Examiner's assertion that the specification provides no information regarding the level of protein expression in tumor and normal tissue, Applicants maintain that, as discussed in more detail below, it is well-established in the art that a change in the level of mRNA for a particular protein, e.g. an increase, generally leads to a corresponding change in the level of the encoded protein.

With respect to the Examiner's assertion that the specification does not disclose the significance of PRO1864 polypeptide expression, Applicants submit that whether or not PRO1864 is the causative agent for melanoma does not impact its use as a diagnostic tool for cancer. One does not need to know what the physiological consequence of the differential expression is in order to exploit the differential expression to distinguish tumor from normal tissue or to utilize antibodies against the claimed polypeptides to treat cancer.

The Examiner maintains that the asserted utility for the claimed polypeptides is based on the presumption that increased mRNA production leads to increased protein production. According to the Examiner, Hu is directly on point by showing that this presumption is incorrect when designating protein as diagnostic markers for cancer. Hu analyzed 2286 genes that showed a greater than 1-fold difference in mean expression level between breast cancer samples and normal samples in a microarray (p. 408, middle of right column) and discovered that, for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a known role in the disease. The Examiner acknowledges that Hu demonstrates that, among genes with a 10-fold or more change in expression level, there was a strong and significant correlation between expression level and a published role in the disease. However, the Examiner asserts that the specification does not disclose that PRO1864 mRNA levels are expressed at 10-fold or higher levels compared with normal, matched tissue samples.

As previously noted, in Hu the researchers used an automated literature-mining tool to summarize and estimate the relative strengths of all human gene-disease relationships published on Medline. They then generated a microarray expression dataset comparing breast cancer and normal breast tissue. Using their data-mining tool, they looked for a correlation between the strength of the literature association between the gene and breast cancer, and the magnitude of the difference in expression level. They report that for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a *known* role in the disease. *See* Hu at 411. However, among genes with a 10-fold or more change in expression level, there was a strong correlation between expression level and a *published* role in the disease. *Id.* at 412. As previously noted, Hu reports that the observed correlation was only found among estrogen receptor-positive tumors, not ER-negative tumors. *Id.*

The general findings of Hu are not surprising – one would expect that genes with the greatest change in expression in a disease would be the first targets of research, and therefore have the strongest known relationship to the disease as measured by the number of publications reporting a connection with the disease. The correlation reported in Hu only indicates that the greater the change in expression level, the more likely it is that there is a *published* or *known* role

for the gene in the disease, as found by their automated literature-mining software. Thus, Hu's results merely reflect a bias in the literature toward studying the most prominent targets, and reflect nothing regarding the ability of a gene that is 2-fold or more differentially expressed in tumors to serve as a disease marker. Hu acknowledges the shortcomings of this method in explaining the disparity in Hu's findings for ER-negative versus ER-positive tumors: Hu attributes the "bias in the literature" toward the more prevalent ER-positive tumors as the explanation for the lack of any correlation between number of publications and gene expression levels in less-prevalent (and, therefore, less studied) ER-negative tumors. *Id.* Because of this intrinsic bias, Hu's methodology is unlikely to ever note a correlation of a disease with less differentially-expressed genes and their corresponding proteins, regardless of whether or not an actual relationship between the disease and less differentially-expressed genes exists. Accordingly, Hu's methodology yields results that provide little or no information regarding biological significance of genes with less than 5-fold expression change in disease.

Furthermore, Applicants note that Hu was examining whether the genes considered in the study had a causative role in disease, not whether they could be used as diagnostic markers. Accordingly, Applicants maintain that Hu is not relevant to the use of the claimed polypeptides as diagnostic markers.

The Examiner cites the LaBaer reference as teaching that reports of mRNA or protein changes of as little as two-fold are not uncommon, and although changes of this magnitude may turn out to be important, most are attributable to disease-independent differences between the samples (emphasis added; 2003, Nature Biotechnology 21(9):976-977).

Applicants note that the LaBaer reference is an unreviewed letter to the editor describing the automated literature searching tool used in the Hu *et al.* reference discussed above. In fact, LaBaer is the last author on the Hu *et al.* reference. Accordingly, LaBaer suffers from the same defects discussed above with respect to Hu *et al.* In particular, the biological correlations referred to in LaBaer are correlations with a *published* or *known* role for the gene in the disease. As discussed above, with respect to Hu *et al.*, the results retrieved by the automated literature searching tool of LaBaer reflect a bias in the literature toward studying the most prominent targets, and reflect nothing regarding the ability of a gene that is 2-fold or more differentially expressed in tumors to serve as a disease marker. Again, because of this intrinsic bias, LaBaer is

unlikely to ever note a correlation of a disease with less differentially-expressed genes and their corresponding proteins, regardless of whether or not an actual relationship between the disease and less differentially-expressed genes exists. Because LaBaer's analysis focuses on whether the genes being assessed have a known role in the disease, it does not address the ability of differentially expressed polypepeptides to serve as diagnostic markers.

In addition, it is important to note that Applicants' are not relying on microarray data as discussed in Hu and LaBaer. Instead, they are relying on a more accurate and reliable method of assessing changes in mRNA level, namely quantitative PCR analysis. In a recent study by Kuo *et al.*, (Proteomics 5(4):894-906 (2005)), the authors used microarray analysis combined with proteomic analysis using two-dimensional gel electrophoresis to examine changes in gene expression in leukemia cell lines, just as discussed in LaBaer. The authors report that "[c]omparison of microarray and proteomic expression profiles showed poor correlation. Use of more reliable and sensitive analyses, such as reverse transcriptase polymerase chain reaction [RT-PCR], Western blotting and functional assays, on several genes and proteins, nonetheless, confirmed that there is indeed good correlation between mRNA and protein expression." Kuo *et al.* at Abstract (emphasis added) (attached as Exhibit 1). Thus, even if accurate, Hu and LaBaer's statements regarding microarray studies are not relevant to the instant application which does not rely on microarray data.

In conclusion, Applicants submit that the evidence reported in Example 18, combined with the first Grimaldi Declaration, establish that there is at least a two-fold difference in PRO1864 cDNA in melanoma compared to normal skin tissue. Therefore, it follows that expression levels of the PRO1864 gene can be used to distinguish melanoma from normal skin tissue. The PTO has not offered any significant arguments or evidence to the contrary.

As Applicants explain below, it is more likely than not that the PRO1864 polypeptide is also differentially expressed in melanoma, and can therefore be used to distinguish melanoma from normal skin tissue. This provides utility for the claimed polypeptides.

Applicants have established that the Accepted Understanding in the Art is that there is a Direct Correlation between mRNA Levels and the Level of Expression of the Encoded Protein

Applicants next turn to the second portion of their argument in support of their asserted utility – that it is well-established in the art that a **change** in the level of mRNA for a particular protein generally leads to a corresponding **change** in the level of the encoded protein; given Applicants' evidence of differential expression of the mRNA for the PRO1864 polypeptide in melanoma, it is more likely than not that the PRO1864 polypeptide is differentially expressed; and proteins differentially expressed in certain tumors have utility as diagnostic or therapeutic tools.

In support of the above position, Applicants submitted a copy of a second Declaration by J. Christopher Grimaldi, an expert in the field of cancer biology (previously submitted as Exhibit 2 with the Amendment and Response to Office Action mailed April 29, 2005). As stated in paragraph 5 of the Declaration, "Those who work in this field are well aware that in the vast majority of cases, when a gene is over-expressed...the gene product or polypeptide will also be over-expressed.... This same principal applies to gene under-expression." Further, the Declaration states "the detection of increased mRNA expression is expected to result in increased polypeptide expression, and the detection of decreased mRNA expression is expected to result in decreased polypeptide expression. The detection of increased or decreased polypeptide expression can be used for cancer diagnosis and treatment." The references cited in the Declaration and submitted therewith support this statement.

Applicants also previously submitted a copy of the Declaration of Paul Polakis, Ph.D. (previously attached as Exhibit 3 with the Amendment and Response to Office Action mailed April 29, 2005), an expert in the field of cancer biology. As stated in paragraph 6 of his Declaration:

> Based on my own experience accumulated in more than 20 years of research, including the data discussed in paragraphs 4 and 5 above [showing a positive correlation between mRNA levels and encoded protein levels in the vast majority of cases] and my knowledge of the relevant scientific literature, it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell. In fact, *it remains a central dogma in molecular biology that increased mRNA levels*

> *are predictive of corresponding increased levels of the encoded protein.* (Emphasis added).

Dr. Polakis acknowledges that there are published cases where such a correlation does not exist, but states that it is his opinion, based on over 20 years of scientific research, that "such reports are exceptions to the commonly understood general rule that increased mRNA levels are predictive of corresponding increased levels of the encoded protein." (Polakis Declaration, paragraph 6).

The statements of Grimaldi and Polakis are supported by the teachings in Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) (previously submitted as Exhibit 4 with the Amendment and Response to Office Action mailed April 29, 2005) and (4th ed. 2002) (previously submitted as Exhibit 5 with the Amendment and Response to Office Action mailed April 29, 2005). Figure 9-2 of Exhibit 4 shows the steps at which eukaryotic gene expression can be controlled. The first step depicted is transcriptional control. Exhibit 4 provides that "[f]or most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized." Exhibit 4 at 403 (emphasis added). In addition, the text states that "Although controls on the initiation of gene transcription are the predominant form of regulation for most genes, other controls can act later in the pathway from RNA to protein to modulate the amount of gene product that is made." Exhibit 4 at 453 (emphasis added). Thus, as established in Exhibit 4, the predominant mechanism for regulating the amount of protein produced is by regulating transcription initiation.

In Exhibit 5, Figure 6-3 on page 302 illustrates the basic principle that there is a correlation between increased gene expression and increased protein expression. The accompanying text states that "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – *most obviously by controlling the production of its mRNA*." Exhibit 5 at 302 (emphasis added). Similarly, Figure 6-90 on page 364 of Exhibit 5 illustrates the path from gene to protein. The accompanying text states that while potentially each step can be regulated by the cell, "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes." Exhibit 5 at 364 (emphasis added). This point is repeated on page 379, where the authors state that of all the possible points for

regulating protein expression, "[f]or most genes transcriptional controls are paramount." Exhibit 5 at 379 (emphasis added).

As previously noted, further support for Applicants' position can be found in the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)) (previously submitted as Exhibit 6 with the Amendment and Response to Office Action mailed April 29, 2005) which states "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added).

Additional support is also found in Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004, previously submitted as Exhibit 7 with the Amendment and Response to Office Action mailed April 29, 2005. Zhigang studied the expression of prostate stem cell antigen (PSCA) protein and mRNA to validate it as a potential molecular target for diagnosis and treatment of human prostate cancer. The data showed "a high degree of correlation between PSCA protein and mRNA expression" Exhibit 7 at 4. Of the samples tested, 81 out of 87 showed a high degree of correlation between mRNA expression and protein expression. The authors conclude that "it is demonstrated that PSCA protein and mRNA overexpressed in human prostate cancer, and that the increased protein level of PSCA was resulted from the upregulated transcription of its mRNA." Exhibit 7 at 6. Even though the correlation between mRNA expression and protein expression occurred in 93% of the samples tested, not 100%, the authors state that "PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor." Exhibit 7 at 7.

Further, Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002), previously submitted as Exhibit 8 with the Amendment and Response to Office Action mailed April 29, 2005, states the following:

> The **fundamental principle** of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells...[M]ost efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable to either DNA amplification or to differences in transcription. Meric *et al.* at 971 (emphasis added).

Those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression.

Together, the declarations of Grimaldi and Polakis, the accompanying references, and the excerpts and references provided above all establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein.

The Examiner asserts that the Declarations of Mr. Grimaldi and Dr. Polakis are not persuasive. The Examiner assumes that the Declarations of Mr. Grimaldi and Dr. Polakis are referring to the central dogma of molecular biology as originally formulated by Crick F. and re-stated in the 1970 Nature publication (Nature 227:561-563, 1970). The Examiner asserts that Crick's central dogma only speaks to the flow of information, i.e., the residue-by-residue transfer of sequential information. The Examiner asserts that there have not been any changes to the central dogma of Crick, such as drawing quantitative correlations between levels of mRNA and protein. The Examiner also asserts that the Declarations do not provide any objective evidence such that the Examiner can independently draw conclusions.

The Grimaldi and Polakis Declarations are not referring to Crick's dogma regarding the direction in which information flows but rather to "the dogma that a change in mRNA will represent a similar change in protein" (Second Grimaldi Declaration, paragraph 5) or the "central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein" (Polakis Declaration, paragraph 6). As discussed in more detail below, the dogma referred to in these Declarations is supported by the Alberts, Lewin, Zhigang and Meric references discussed above, which demonstrate that the predominant point at which the expression of polypeptides is regulated is transcription.

The Examiner asserts that Alberts and Lewin support the position that further research would have to be carried out to determine if the polypeptide expression levels track with the expression levels of the corresponding mRNA. According to the Examiner, Alberts and Lewin show that there are several levels that control gene expression both at the transcriptional (i.e., mRNA synthesis) and the translational (i.e., protein production) levels. The Examiner states that it is important to note that transcription occurs in the nucleus, whereas translation occurs in the

cytoplasm and that one skilled in the art would not accept that increased mRNA levels directly correlate with the level of the corresponding polypeptide in view of the multitude of controls at the transcriptional and translational levels. In addition, the Examiner asserts that, while increased transcript levels can lead to increased polypeptide levels, there are other regulatory factors that also effect the rate of translation as evidenced by Alberts (3rd Ed.) in Figure 9-72.

Applicants acknowledge that gene regulation may occur at a point other than transcription initiation. However, the PTO does not address the teachings in Alberts (3rd ed., previously submitted as Exhibit 4 with the Amendment and Response to Office Action mailed April 29, 2005, that "[f]or most genes transcriptional controls are paramount" and that "[c]ontrols on the initiation of gene transcription are the predominant form of regulation for most genes." Exhibit 4 at 403 and 453 (emphasis added). Likewise, the PTO does not address the teachings in Alberts (4th ed., previously submitted as Exhibit 5 with the Amendment and Response to Office Action mailed April 29, 2005) that "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes. Exhibit 5 at 364 (emphasis added). Like Exhibit 4 discussed above, Exhibit 5 also states that "[f]or most genes transcriptional controls are paramount." Exhibit 5 at 379 (emphasis added). Furthermore, while Figure 6-3 indicates that different genes can be transcribed and translated with different efficiencies, the text following the reference to Figure 6-3 states "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – most obviously by controlling the production of its mRNA." Exhibit 5 at 302 (emphasis added). Accordingly, Applicants submit that full consideration of this evidence would lead to a conclusion that mRNA levels are paramount in influencing polypeptide expression levels.

With respect to Lewin, Applicants maintain that the PTO does not address the teachings in Lewin that "the overwhelming majority of regulatory events occur at the initiation of transcription." Exhibit 6 submitted with the Amendment and Response to Office Action mailed April 29, 2005 (emphasis added). Again, Applicants submit that full consideration of this evidence would lead to a conclusion that mRNA levels are paramount in influencing polypeptide expression levels.

Although the Examiner agrees that statistical certainty is not the standard to establish an asserted utility, he asserts that the experiments of Zhigang evince that one needs to actually determine the expression of the protein to be sure of expression.

Zhigang reported that the correlation between mRNA expression and protein expression occurred in 93% of the samples tested. As discussed above, and acknowledged by the Examiner, Applicants submit that there is no requirement to provide evidence sufficient to establish an asserted utility as a matter of statistical certainty. "Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true." M.P.E.P. at § 2107.02, part VII (2004) (emphasis in original, internal citations omitted). However, the PTO holds that a showing of 93% correlation between mRNA and polypeptide levels is an indication of support for unpredictability of the relationship between mRNA and polypeptide levels. Thus, it is clear from the PTO's evaluation of the PTO's evidence and Applicants' evidence that the PTO is requiring that Applicants establish their asserted utility as a matter of statistical certainty. This standard is inconsistent with the Utility Guidelines and governing case law. Since the PTO's rejection of the claimed polypeptides as lacking utility is based on this incorrect standard, the basis for holding of a lack of utility cannot be maintained.

Additionally, the Examiner asserts that Meric et al. teaches that, in addition to variations in mRNA sequences that increase or decrease translational efficiency, changes in the expression or availability of components of the translational machinery as well as activation of translation through aberrantly activated signal transduction pathways also effect the rate of translation in cancerous cells. According to the Examiner, Meric et al. in agreement with Alberts and Lewin acknowledges that gene expression is quite complicated and is regulated at the level of mRNA stability, mRNA translation and protein stability. The Examiner also asserts that Meric goes on to discuss that the components of the translation machinery and signal pathways involved in the activation of translation initiation represent good targets for cancer therapy. According to the Examiner, if it is the accepted understanding in the art that there is a direct correlation between mRNA levels and the level of expression of the encoded polypeptide, there would not be a need to target the translational machinery, unless of course the two are separate.

As noted above, Applicants have already acknowledged that gene expression is regulated at numerous levels. However, as the supporting references and Declarations Applicants have supplied make clear, regulation of mRNA levels is the predominant mechanism of control for the majority of genes. Meric supports this assertion because "[t]he **fundamental principle** of molecular therapeutics in cancer is to exploit the **differences** in gene expression between cancer cells and normal cells." Meric *et al.* at 971 (emphasis added). The only reason mRNA is of any interest in studying the mechanism of cancer formation and growth is because mRNA encodes protein. If there were no general correlation between differences in mRNA and differences in protein, there would be no reason to study changes in mRNA. Furthermore, with respect to the Examiner's argument that there would be no need to target translational machinery for cancer therapy if there was a direct correlation between mRNA and protein, Applicants maintain that any point in the process of producing a polypeptide involved in cancer may be exploited as a target for therapy. The inclusion of translational machinery amongst the many potential target points does not indicate in any way that there is no correlation between mRNA levels and polypeptide levels.

The Examiner reiterates his previous rejection asserting that Gokman-Polar indicates that mRNA levels do not necessarily correlate with protein levels and that expression may be regulated at the posttranscriptional/translational level.

Applicants continue to maintain that a close review of Gokman-Polar indicates that, with one exception, the trend in the data is that mRNA and protein levels are positively correlated, supporting Applicants assertion that increased mRNA levels correlate with increased protein levels. Applicants again note that, in Figure 2, the protein level of two isozymes shows a decrease, while the third is increased. This same pattern is seen for the corresponding mRNA levels in Figure 6, although admittedly the increase in mRNA for the third isozyme is minimal. Similarly, comparing the protein levels of the three isozymes in Figure 4 to the corresponding mRNA levels in Figure 7, with one exception the mRNA levels are positively correlated to protein levels. While protein levels do not increase or decrease in direct proportion to the changes in mRNA, the trend in five of the six examples is that protein levels are positively correlated to mRNA levels. Accordingly, Applicants submit that this evidence is hardly

sufficient to establish that one of skill in the art would reasonably doubt that there is a reasonable correlation between mRNA levels and protein levels.

The Examiner also reiterates his previous assertion that that Gygi et al. found that even similar mRNA expression levels could be accompanied by a wide range (up to 20-fold difference) of protein abundance levels, and vice versa. In response to Applicants previous arguments, the Examiner asserts that Applicants criticize Gygi by stating that Gygi has nothing to do with changes in protein levels resulting from changes in mRNA levels. The Examiner then asserts that Applicants are holding Gygi to a higher standard than their own specification, which provides no evidence or correlation between increased PRO1864 mRNA levels and increased PRO1864 protein levels. The Examiner further maintains that Gygi supports the Examiner's position which that there is no necessary correlation between mRNA levels and protein levels and one cannot predict protein levels from mRNA. The Examiner also cites Haynes et al., which refers to the data in the Gygi reference, as showing that, for some genes, equivalent mRNA levels translated into protein abundances which varied more than 50-fold. The Examiner asserts that Haynes et al. concluded that the protein levels cannot be accurately predicted from the level of the corresponding mRNA transcript.

As previously noted, Haynes and Gygi do not contradict the utility of the instant claims. Applicants continue to maintain that Gygi found that "there was a general trend of increased protein levels resulting from increased mRNA levels." Gygi p. 1726. However, neither Gygi nor Haynes looked at whether a change in transcript level for a particular gene led to a change in the level of expression of the corresponding protein. Instead, these references considered whether the steady-state transcript level correlated with the steady-state level of the corresponding protein. These references disclose that similar mRNA levels for *different* genes did not universally result in equivalent protein levels for the *different* gene products, and similar protein levels for *different* gene products did not universally result from equivalent mRNA levels for the *different* genes. These results are expected, since there are many factors that determine translation efficiency for a given transcript, or the half-life of the encoded protein. Not surprisingly, based on these results, Haynes concluded that protein levels cannot always be accurately predicted from the level of the corresponding mRNA transcript *when looking at the level of transcripts across different genes.*

Importantly, Haynes and Gygi did not say that for a single gene, the level of mRNA transcript is not positively correlated with the level of protein expression. Applicants maintain that increasing or decreasing the level of mRNA for the same gene leads to an increase or decrease for the corresponding protein. Haynes and Gygi did not study this issue and say absolutely nothing about it. One cannot look at the level of mRNA across several different genes to investigate whether a change in the level of mRNA a particular gene leads to a change in the level of protein for that gene. Haynes and Gygi have nothing to do with changes in protein levels resultant from changes in mRNA levels because they did not examine whether a change in transcript level for a particular gene led to a change in the level of expression of the corresponding protein. Thus, when these references assert that protein levels cannot be accurately predicted from mRNA levels, this refers to the finding that cellular protein levels cannot always be calculated simply based on cellular mRNA levels. This is completely unrelated to the expectation of a change in protein levels as a function of the change in encoding mRNA levels. Neither Haynes nor Gygi provide any insight whatsoever into the affects on protein levels caused by a change in the encoding mRNA levels. Applicants continue to assert that increasing or decreasing the level of mRNA for the same gene leads to an increase or decrease for the corresponding protein. Since this issue is not addressed in Haynes and Gygi, these references offer no support for the PTO's position.

The Examiner cites Greenbaum et al. as cautioning against assuming that mRNA levels are generally correlative of protein levels. According to the Examiner, this reference teaches that, primarily because of a limited ability to measure protein abundance, researchers have tried to find correlations between mRNA and the limited protein expression data, in the hope that they could determine protein abundance levels from the more copious and technically easier mRNA experiments. According to the Examiner, this reference further teaches that there are presumably at least three reasons for the poor correlations generally reported in the literature between the level of mRNA and the level of protein, and these may not be mutually exclusive. First, the Examiner asserts that there are many complicated and varied post-transcriptional mechanisms involved in turning mRNA into protein that are not yet sufficiently well defined to be able to compute protein concentrations from mRNA. Second, the Examiner asserts that proteins may differ substantially in their in vivo half lives. Third, the Examiner asserts that there is a

significant amount of error and noise in both protein and mRNA experiments that limit our ability to get a clear picture. According to the Examiner, Greenbaum further notes that to be fully able to understand the relationship between mRNA and protein abundance, the dynamic processes involved in protein synthesis and degradation have to be better understood.

In contrast to the Examiner's position, Applicants maintain that Greenbaum demonstrates that, for genes in which the level of mRNA expression varies, there is a high degree of correlation between mRNA and protein levels. In particular, Greenbaum states:

> Logically, we would assume that those ORFs that show a large degree of variation in their expression are controlled at the transcriptional level-the variability of the mRNA expression is indicative of the cell controlling mRNA expression at different points of the cell cycle to achieve the resulting and desired protein levels. Thus we would expect, and we found, a high degree of correlation (r=0.89) between the reference mRNA and protein levels for these particular ORFs; the cell has already put significant energy into dictating the final level of protein through tightly controlling the mRNA expression, and we assume that there would then be minimal control at the protein level.

With respect to genes which do not show variation at the mRNA level, Greenbaum's lack of correlation is analogous to that discussed above with respect to the Haynes and Gygi references. Again, as discussed above, one cannot look at the level of mRNA across several different genes to investigate whether a change in the level of mRNA a particular gene leads to a change in the level of protein for that gene. Furthermore, as discussed above, Applicants are not asserting that transcriptional regulation is the only mechanism for controlling protein levels. Rather, Applicants maintain that transcriptional regulation is the predominant mechanism for regulating protein levels.

The Examiner cites Lian et al. as showing a lack of correlation between mRNA and protein in mammalian cells. In Lian, the authors looked at the mRNA and protein levels of genes in a derived promyelocytic mouse cell-line during differentiation of the cells from a promyelocytic stage of development to mature neutrophils following treatment with retinoic acid. *Lian* at Abstract. The level of mRNA expression was measured using 3'-end differential display (DD) and oligonucleotide chip array hybridization, and protein levels were qualitatively assessed following 2-dimensional gel electrophoresis. *Id.* at Abstract, Table 6.

Lian *et al.* used DD and array hybridization to examine the expression of genes 0, 24, 48 and 72 hours after treatment with retinoic acid. *Id.* at 515, col. 1, ¶ 2. Using this information, the authors constructed a database of mRNA level changes during differentiation of the cell line. *Id.* at 518, col. 2, ¶ 2. Lian *et al.* also examined protein expression at 0 and 72 hours after retinoic acid treatment. Lian reports that they were able to identify 28 proteins which they considered differentially expressed. *Id.* at 521, Fig. 5. Of those 28, only 18 had corresponding gene expression information in the database, and only 13 had measurable levels of mRNA expression. *Id.* at 521, Table 6. The authors then compared the qualitative protein level from the 2-D electrophoresis gel to the corresponding mRNA level, and reported that only 4 genes of the 18 present in the database had expression levels which were consistent with protein levels. *Id.* at 512, col. 1. The authors note that "[n]one of these was on the list of genes that were differentially expressed significantly (5-fold or greater change by array or 2-fold or greater change by DD)." *Id.* at 512, bridge paragraph (emphasis added). Based on these data, the authors conclude "[f]or protein levels based on estimated intensity of Coomassie dye staining in 2DE, there was poor correlation between changes in mRNA levels and estimated protein levels." *Id.* at 522, col. 2, ¶ 2.

These results are not contrary to Applicants' assertion. Applicants emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. Based on the authors' criteria, mRNA levels were significantly changed if they were at least 5-fold different when measured using a microchip array, or 2-fold different when using the more sensitive 3'-end differential display (DD). Of the 28 proteins listed in Table 6, only one has an mRNA level measured by microarray which is differentially expressed according to the authors (spot 7: melanoma X-actin, which mRNA changed from 2539 to 341.3, and protein changed from 1 to 3). None of the other mRNAs listed in Table 6 show a significant change in expression level when using the criteria established by the authors for the less sensitive microarray technique.

There is also one gene in Table 6 whose expression was measured by the more sensitive technique of DD, and its level increased from a qualitative value of 0 to 2, a more than 2-fold

increase (spot 2: actin, gamma, cytoplasmic). This increase in mRNA was accompanied by a corresponding increase in protein level, from 3 to 6.

Therefore, although the authors characterize the mRNA and protein levels as having a "poor correlation," this does not reflect a lack of a correlation between a change in mRNA level and a corresponding change in protein level. Only two genes meet the authors' criteria for differentially expressed mRNA level, and of those, one apparently shows a corresponding change in protein level and one does not. *Id.* at 521, Table 6. Thus, this reference does not contradict Applicants' position that, in general, a change in mRNA level corresponds to a change in the level of the encoded polypeptide.

The Examiner also cites Fessler et al. as showing a "[p]oor concordance between mRNA transcript and protein expression changes" in human cells. (Fessler et al., page 31291, abstract).

Fessler *et al.* studied changes in neutrophil (PMN) gene transcription and protein expression following lipoplysaccharide (LPS) exposure. Fessler lists in Table VIII a comparison of the change in the level of mRNA for 13 up-regulated proteins and 5 down-regulated proteins. Of the 13 up-regulated proteins, a change in mRNA levels is reported for only 3 such proteins. For these 3, mRNA levels are increased in 2 and decreased in the third. Of the 5 down-regulated proteins, a change in mRNA is reported for 3 such proteins. In all 3, mRNA levels also are decreased. Thus, in 5 of the 6 cases for which a change in mRNA levels are reported, the change in the level of mRNA corresponds to the change in the level of the protein. This is consistent with Applicants assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Regarding the remainder of the proteins listed in Table VIII, in 6 instances, protein levels changed while mRNA levels were unchanged. This evidence has no relevance to Applicants' assertions of the influence that changes in mRNA levels have on protein levels. In explaining these instances, Fessler explains that LPS has post-transcriptional activity that can influence protein levels (Fessler at 31300, right column). Nothing in these results by Fessler suggests that a change in the level of mRNA for a particular protein does not generally lead to a corresponding change in the level of the encoded protein. Accordingly, these results are not contrary to Applicants assertions.

In the remaining 6 instances listed in Table VIII, protein levels changed while mRNA was noted as "absent." This evidence also has no relevance to Applicants' assertions of the influence that changes in mRNA levels has on protein levels. By virtue of being "absent," it is not possible to tell whether mRNA levels were increased or decreased in PMN upon contact with LPS. Regarding these instances, Fessler explained that LPS may have post-translational activity that can result in increased protein stability (Fessler at 31300, right column). Nothing in these results by Fessler suggests that a change in the level of mRNA for a particular protein does not generally lead to a corresponding change in the level of the encoded protein. Accordingly, these results also are not contrary to Applicants assertions.

The PTO points to Fessler's statement regarding Table VIII that "a poor correlation was found between corresponding transcripts and proteins." (Fessler at 31300, right column). As is clear from the above discussion, this statement does not relate to a lack of correlation of a change in mRNA levels and protein levels, because in 5 of 6 such instances, changes in mRNA and protein levels correlated well. Instead, this statement relates to observations in which protein levels changed when mRNA was either unchanged or absent. As such, this statement is an observation that in addition to transcriptional activity, LPS also has post-transcriptional and possibly post-translational activity that affect protein levels. Thus, Fessler's results suggest that LPS has a transcriptional activity that can cause changes in protein levels which correlate with changes in mRNA levels, and LPS also has post-transcriptional activity that can cause changes in protein levels that do not correlate with unchanged or absent mRNA levels. Accordingly, Fessler's results are consistent with Applicants assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Even if Fessler's results had shown that a change in the level of mRNA did not generally lead to a corresponding change in the level of the encoded protein, which they did not, the accuracy of Fessler's results is uncertain. Fessler admits that there were "limitations" to the results reported. These limitations included: possible artifactual transcript-protein discordance due to a 4 hour delay in harvesting after LPS exposure; uncertain post-incubation but pre-electrophoresis effects on protein synthesis, degranulation and exocytosis; and limited ability to quantitate protein amounts using Coomassie Blue. (Fessler at 31301, left column). Fessler exemplifies one such spurious result, in which there was a disparity between observed increase

in cytokine mRNA, but an absence of detected cytokine proteins, which, as Fessler explains, "reflects their removal in the post-LPS incubation wash." (Fessler at 31297, right column). Thus, Fessler acknowledges "limitations" to the conclusion that, for some genes, transcript levels did not coincide well with corresponding protein levels, leaving it uncertain the extent to which actual changes in protein levels differed from mRNA levels when neutrophils were exposed to LPS. As such, Fessler does not contradict Applicants' position that, in general, a change in mRNA level correlates with a change in the level of the encoded polypeptide. Instead, Fessler represents a teaching that LPS might cause transcriptional changes that correlate with changes in protein levels, and might also cause post-transcriptional changes in protein levels when mRNA levels are unchanged. Accordingly, Fessler is not contrary to Applicants' asserted utility.

The Examiner also cites Hanash S. [a] (Nature Reviews, Applied Proteomics Collection, pp. 9-14, March 2005) as teaching that "There is a need to profile gene expression at the level of the proteome and to correlate changes in gene-expression profiles with changes in proteomic profiles. The two are not always linked-numerous alterations occur in protein levels that are not reflected at the RNA level." (Hanash, S [a], page 12). The Examiner also asserts that Hanash [a] teaches that tumors are complex biological systems and no single type of molecular approach fully elucidates tumor behavior, necessitating analysis at multiple levels encompassing genomics and proteomics. The Examiner also cites the statement in Hanash et al. [b] (The Pharmacogenomics Journal, 3(6):308-311, 2003) that "However perfected DNA microarrays and their analytical tools become for disease profiling, they will not eliminate a pressing need for other types of profiling technologies that go beyond measuring RNA levels, particularly for disease-related investigations." (Hanash et al. [b], page 311). According to the Examiner, Hanash et al. [b] teaches that there is a need to assay protein levels and activities and that numerous alterations may occur in proteins that are not reflected in changes at the RNA level.

As discussed above, Applicants have already acknowledged that gene expression is regulated at numerous levels. However, as discussed above, the Declarations and supporting references supplied by Applicants make it clear that regulation of mRNA levels is the predominant mechanism of control for the majority of genes.

Applicants' additional supporting references

In addition to the references discussed above which were previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a comprehensive study by Orntoft *et al.* (Mol. Cell. Proteomics. 2002; 1(1):37-45) (previously submitted with IDS, attached hereto as Exhibit 2), the authors examined gene amplification, mRNA expression level, and protein expression in pairs of non-invasive and invasive human bladder tumors. *Id.* at Abstract. The authors examined 40 well resolved abundant known proteins, and found that "[i]n general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations. Only one gene showed disagreement between transcript alteration and protein alteration." *Id.* at 42, col. 2. The alternations in mRNA and protein included both increases and decreases. *Id.* at 43, Table II. Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (abstract attached as Exhibit 3) the authors report that down-regulation of E-cadherin protein has been shown in various human tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and nonneoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (abstract attached as Exhibit 4) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells.

Id. at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 5) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract. The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1 mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 6) the authors report that atrophy of skeletal muscle is common in patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased

protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (abstract attached as Exhibit 7) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 8). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the hippocampal CA region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

Mizrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 9) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in

pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 10), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. ... The loss of SERCA protein expression is mediated by down-regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level lead to a corresponding change in the level of the encoded protein.

In an article by Guo and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 11) the authors investigated the expression of macrophage migration inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, e.g. an increase, generally leads to a corresponding change in the level of protein expression, e.g. an increase.

These studies are representative of numerous published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an additional 70 references (abstracts attached as Exhibit 12) which support Applicants' assertion.

In addition to these supporting references, Applicants also submit herewith additional references which offer indirect support of Applicants' asserted utility. As discussed above, Applicants have challenged the relevance of references such as Haynes *et al.* and Gygi *et al.*, which do not attempt to examine the correlation between a change in mRNA level and a change in the level of the corresponding protein level. Because the PTO continues to rely on these references, Applicants are submitting references which report results that are contrary to the PTO's cited references and offer indirect support for Applicants' asserted utility.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (abstract attached as Exhibit 13) the authors conducted a study of mRNA and protein expression in yeast which was nearly identical to the one conducted by Gygi *et al.* Contrary to the results of the earlier study by Gygi, Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

In a study which is more closely related to Applicants' asserted utility, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 15) the authors examined the expression of three somatostatin receptors (SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 16) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 17) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 18) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases)." *Id.* at Abstract. "There was a good correlation between cyclin D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the PTO's arguments. Applicants submit herewith 26 additional references (abstracts attached as Exhibit 20) which also support Applicants' assertion in that they report a correlation between the level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

In summary, Applicants submit herewith a total of 114 references in addition to the Declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references support the assertion that, in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.,* abstracts attached as Exhibit 21). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for

rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

In conclusion, Applicants submit that they have offered sufficient evidence to establish that it is more likely than not that one of skill in the art would believe that because the PRO1864 mRNA is more highly expressed in melanoma compared to normal skin tissue, the PRO1864 polypeptide will likewise be differentially expressed in melanoma. This differential expression of the PRO1864 polypeptide makes the claimed polypeptides useful as diagnostic tools or therapeutic tools for cancer, particularly melanoma.

The Arguments made by the PTO are Not Sufficient to satisfy the PTO's Initial Burden of Offering Evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility"

Applicants continue to maintain that an assertion of utility in the specification creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297 (CCPA 1974). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the evidence, or "more likely than not" standard. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). This is stated explicitly in the M.P.E.P.:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. M.P.E.P. at § 2107.02, part VII (2004) (underline emphasis in original, bold emphasis added, internal citations omitted).

The PTO has the initial burden to offer evidence "that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). Only then does the burden shift to the Applicant to provide rebuttal evidence. *Id.* As stated in the M.P.E.P., such rebuttal evidence does not need to absolutely prove

that the asserted utility is real. Rather, the evidence only needs to be reasonably indicative of the asserted utility.

Applicants continue to maintain that the PTO has not offered any arguments or cited any references to establish "that one of ordinary skill in the art would reasonably doubt" that it is more likely than not that the disclosed polypeptide is differentially expressed in certain tumors and that the claimed polypeptides can be used as diagnostic and therapeutic tools. Given the lack of support for the PTO's position, Applicants submit that the PTO has not met its initial burden of overcoming the presumption that the asserted utility is sufficient to satisfy the utility requirement. And even if the PTO has met that burden, the Applicants' supporting rebuttal evidence is sufficient to establish that one of skill in the art would be more likely than not to believe that the claimed polypeptides can be used as diagnostic agents for cancer, particularly melanoma.

Specific Utility

The Asserted Substantial Utilities are Specific to the Claimed Polypeptides

Applicants continue to maintain that the asserted utility is specific to the PRO1864 polypeptide. Specific utility is defined as utility which is "specific to the subject matter claimed," in contrast to "a general utility that would be applicable to the broad class of the invention." M.P.E.P. § 2107.01 I. Applicants submit that the evidence of differential expression of the PRO1864 gene and polypeptide in certain types of tumor cells, along with the declarations and references discussed above, provide a specific utility for the claimed polypeptides.

As discussed above, there are significant data which show that the gene for the PRO1864 polypeptide is more highly expressed in melanoma compared to normal skin tissue. These data are strong evidence that the PRO1864 gene and polypeptide are associated with melanoma. Thus, Applicants submit that they have provided evidence associating the PRO1864 gene and polypeptide with a specific disease. The asserted utility as a diagnostic and therapeutic tool for cancer, particularly melanoma, is a specific utility – it is not a general utility that would apply to the broad class of polypeptides.

Conclusion

The PTO has asserted three arguments to support its conclusion that the differential expression of PRO1864 is not sufficient to establish utility for the claimed polypeptides.

1. The PTO has challenged the reliability of the evidence reported in Example 18 and states that the specification does not teach the expression of the PRO1864 protein, any particular biological activity of the polypeptide, or the levels of the PRO1864 polypeptide in tumor samples relative to normal samples.

2. The PTO cites Hu *et al* and LaBaer *et al.* in support of its position that the differential mRNA expression data in Example 18 is insufficient to convey utility to the claimed polypeptides;

3. The PTO cites Crick, Gökman-Polar, Gygi, Greenbaum , Haynes, Lian et al., Fessler et al., Hanash [a], and Hanash [b] in support of its position that there is no correlation between mRNA levels and polypeptide levels. The PTO further maintains that Molecular Biology of the Cell, Lewin, Zhigang, and Meric support this position.

Applicants have provided a first Declaration of Chris Grimaldi stating that the data in Example 18 are real and significant. Applicants have pointed out that the substantial utilities described above are specific to the claimed polypeptides because the PRO1864 gene and polypeptide are differentially expressed in certain cancer cells compared to the corresponding normal cells. This is not a general utility that would apply to the broad class of polypeptides.

Second, Applicants have demonstrated that Hu's results are focused on the correlation between genes and a published role in disease and reflect a bias in the literature toward studying the most prominent targets. As discussed above, Hu's results reflect nothing regarding the ability of a gene that is 2-fold or more differentially expressed in tumors to serve as a disease marker. Applicants have also demonstrated that LaBaer suffers from the same defects as Hu.

Third, Applicants have shown that the second Grimaldi Declaration and Polakis Declaration, the accompanying references, as well as the excerpts and references cited above, demonstrate that it is well-established in the art that a change in mRNA levels generally correlates to a corresponding change in the encoded protein levels. Applicants have shown that the references cited by the PTO do not contradict the general rule that changes in mRNA level correlate with changes in the level of the encoded polypeptide. One of skill in the art will

recognize that polypeptides differentially expressed in certain cancers have utility as diagnostic tools for cancer.

Given the totality of the evidence provided, Applicants submit that they have established a substantial, specific, and credible utility for the claimed polypeptides as diagnostic and therapeutic tools. According to the PTO Utility Examination Guidelines (2001), irrefutable proof of a claimed utility is not required. Rather, a specific, substantial, and credible utility requires only a "reasonable" confirmation of a real world context of use. Applicants remind the PTO that:

> A small degree of utility is sufficient . . . The claimed invention must only be capable of performing some beneficial function . . . An invention does not lack utility merely because the particular embodiment disclosed in the patent lacks perfection or performs crudely . . . A commercially successful product is not required . . . Nor is it essential that the invention accomplish all its intended functions . . . or operate under all conditions . . . partial success being sufficient to demonstrate patentable utility . . . In short, **the defense of non-utility cannot be sustained without proof of total incapacity**. If an invention is only partially successful in achieving a useful result, a rejection of the claimed invention as a whole based on a lack of utility is not appropriate. M.P.E.P. at 2107.01 (underline emphasis in original, bold emphasis added, citations omitted).

Applicants submit that they have established that it is more likely than not that one of skill in the art would reasonably accept the utility for the claimed polypeptides related to PRO1864 set forth in the specification. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Enablement

Claims 4-17 were rejected under 35 U.S.C. §112, first paragraph, on the assertion that, since the claimed invention is not supported by a substantial utility or a well-established utility, one skilled in the art would not know how to use it. Claims 4-17 were also rejected under 35 U.S.C. §112, first paragraph, on the assertion that the specification does not enable one skilled in the art to practice the claimed invention because the art of record discussed above with respect to the utility rejections underscores the unpredictability in the art. The Examiner asserts that the mRNA expression data does not support the claimed utility because a multitude of homeostatic factors affect transcription and translation.

As discussed above, Applicants maintain that the claimed polypeptides possess specific, substantial and credible utility. With respect to the Examiner's assertion that a number of homeostatic factors affect transcription and translation, as discussed above, Applicants acknowledge that gene expression is regulated at numerous levels. However, as discussed above, the Declarations and supporting references supplied by Applicants make it clear that regulation of mRNA levels is the predominant mechanism of control for the majority of genes. Accordingly, Applicants maintain that one of skill in the art would know how to use the claimed invention.

The Examiner asserts that Applicants have not provided sufficient guidance to assist one skilled in the art to make and use the claimed protein variants that are 95% or 99% identical to SEQ ID NO:14, much less variants that are 95% or 99% identical to amino acids 21-53, 119-129 or 167-234 of SEQ ID NO:14, in a manner reasonably correlated with the scope of the claims, which include any number of additions, deletions, or substitutions and fragments. According to the Examiner, the specification does not teach the biological function of the claimed polypeptides and one of skill in the art would not know how to use the claimed polypeptides or screen for the same.

Claims 4-17 recite polypeptides having at least 95% amino acid sequence identity to the specified sequences related to SEQ ID NO: 14. In view of this high degree of homology, Applicants maintain that the disclosure of the polypeptide of SEQ ID NO: 14 is sufficient to enable one skilled in the art to use the claimed polypeptides. Applicants note that the specification contains extensive discussion regarding the claimed polypeptides. In particular, the specification discloses how to make the claimed polypeptides, for example in Paragraphs [0283]-[0309], [0313]-[0315] and Examples 6-9 of the specification. In addition, variant polypeptides and variant polynucleotides having homology to SEQ ID NO:14 and methods of making them are described in the specification at Paragraphs [0199]-[0220] and Paragraphs [0256]-[0271] of the specification and, in addition, methods of making variant polypeptides are well known in the art. Methods for making and testing antibodies for specificity are well known in the art, and are disclosed in the specification, at Paragraphs [0361]-[0405] and Paragraph [0493]. Methods for using the antibodies as diagnostic agents are well known in the art and are disclosed in the specification at Paragraph [0407]. In particular, the specification discloses use of the antibodies

in diagnostic assays to detect differential expression. *See Specification* at Paragraph [0407]. Antibodies are also discussed in Paragraphs [0225], [0238]-[0247] and [0310]-[0312]. In addition, Example 18 describes the measurement of mRNA levels using PCR. Furthermore, Applicants maintain that the determination of whether a polypeptide is more highly expressed in melanoma compared to normal skin tissue or whether a polypeptide is encoded by a polynucleotide which is more highly expressed in melanoma compared to normal skin tissue involves routine methodology such as Western blotting, Northern Blotting or PCR. The implementation of routine techniques does not constitute undue experimentation. (See *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988). Applicants maintain that the specification enables one skilled in the art to make and use the claimed invention.

The Examiner asserts that one skilled in the art could not use the claimed invention because the specification does not describe any biological activity of the claimed polypeptides. Applicants maintain that the utility of the claimed polypeptides is not dependent on their biological function. In particular, the utility of the claimed polypeptides is a consequence of their differential expression in melanoma compared to normal skin tissue. One does not need to know what the consequence of the differential expression is in order to exploit this differential expression to distinguish tumor from normal tissue or to utilize antibodies against the claimed polypeptides to treat cancer.

The Examiner further asserts that, due to the large quantity of experimentation necessary to generate the large number of protein variants recited in the claims and to screen same for activity, the lack of direction/guidance presented in the specification regarding which structural features are required in order to provide activity, the absence of working examples directed to same, and the complex nature of the invention, undue experimentation would be required to practice the claimed invention. According to the Examiner, the prior art as exemplified by Burgess et al., Lazar et al., Schwartz et al., Lin et al. and Li et al., previously cited in the Office Action mailed 1/31/2005, establishes the unpredictability of the effects of mutation on protein structure and function. The Examiner also asserts that, in view of the breadth of the claims and the lack of structural or functional limitations, undue experimentation would be required to make and use the claimed invention.

Applicants reiterate that, as discussed in more detail below with respect to the written description rejection, there is not substantial variation within the sequences of the polypeptides encompassed by the claims. In addition, as previously noted, the PTO has issued many patents containing claims to variant nucleic acids or variant proteins where the applicants did not actually make such nucleic acids or proteins. Furthermore, Applicants note that, in view of the high degree of homology to the polypeptide of SEQ ID NO: 14 possessed by the claimed polypeptides, it is likely that, even if a particular mutation impacts a polypeptide's biological activity, one or more antigenic epitopes would be preserved such that antibodies raised against the variant polypeptide would recognize the polypeptide of SEQ ID NO: 14 and could be used as diagnostic tools for melanoma. As discussed above, those skilled in the art can make the claimed variant polypeptides, generate antibodies against them, and assess the ability of the antibodies to bind to the polypeptide of SEQ ID NO: 14 using conventional methodology. Accordingly, Applicants maintain that the specification enables those skilled in the art to make and use the claimed invention.

Written Description

Claims 4-5 and 12-17 were rejected under 35 U.S.C 112, first paragraph on the assertion that they fail to comply with the written description requirement.

The Legal Standard for Written Description

As previously stated, the well-established test for sufficiency of support under the written description requirement of 35 U.S.C. §112 , first paragraph is whether the disclosure "reasonably conveys to artisan that the inventor had possession at that time of the later claimed subject matter." *In re Kaslow*, 707 F.2d 1366, 1375, 2121 USPQ 1089, 1096 (Fed. Cir. 1983); *see also Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991). The adequacy of written description support is a factual issue and is to be determined on a case-by-case basis. *See e.g., Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991). The factual determination in a written description analysis depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure. *Union Oil v. Atlantic Richfield Co.*, 208 F.3d 989, 996 (Fed. Cir. 2000).

The Current Invention is Adequately Described

As noted above, whether the Applicants were in possession of the invention as of the effective filing date of an application is a factual determination, reached by the consideration of a number of factors, including the level of knowledge and skill in the art, and the teaching provided by the specification. The inventor is not required to describe every single detail of his/her invention. An Applicant's disclosure obligation varies according to the art to which the invention pertains. The present invention pertains to the field of recombinant DNA/protein technology. It is well-established that the level of skill in this field is very high since a representative person of skill is generally a Ph.D. scientist with several years of experience. Accordingly, the teaching imparted in the specification must be evaluated through the eyes of a highly skilled artisan as of the date the invention was made.

As discussed above, Claims 4-5 and 12-17 recite polypeptides having at least 95% amino acid sequence identity to the specified sequences related to SEQ ID NO: 14. In view of this high degree of homology, Applicants maintain that the disclosure of the polypeptide of SEQ ID NO: 14 is sufficient to describe the claimed polypeptides. Applicants note that the specification contains extensive discussion regarding the claimed polypeptides. In particular, as discussed above, the specification discloses how to make the claimed polypeptides, for example in Paragraphs [0283]-[0309], [0313]-[0315] and Examples 6-9 of the specification. In addition, variant polypeptides and variant polynucleotides having homology to SEQ ID NO:14 and methods of making them are described in the specification at Paragraphs [0199]-[0220] and Paragraphs [0256]-[0271] of the specification and, in addition, methods of making variant polypeptides are well known in the art. Methods for making and testing antibodies for specificity are well known in the art, and are disclosed in the specification, at Paragraphs [0361]-[0405] and Paragraph [0493]. Methods for using the antibodies as diagnostic agents are well known in the art and are disclosed in the specification at Paragraph [0407]. In particular, the specification discloses use of the antibodies in diagnostic assays to detect differential expression. *See Specification* at Paragraph [0407]. Antibodies are also discussed in Paragraphs [0225], [0238]-[0247] and [0310]-[0312]. In addition, Example 18 describes the measurement of mRNA levels using PCR. Furthermore, Applicants maintain that the determination of whether a polypeptide is more highly expressed in melanoma compared to normal skin tissue or whether a polypeptide is

encoded by a polynucleotide which is more highly expressed in melanoma compared to normal skin tissue involves routine methodology such as Western blotting, Northern Blotting or PCR. As discussed above, in light of the differential expression of mRNA encoding the PRO1864 polypeptide in melanoma, one of skill in the art would expect the PRO1864 polypeptide to be differentially expressed in these tumors as well.

The Examiner asserts that, unlike Example 14 of the written description training materials, which encompasses a genus of molecules having significant structural similarity and identical biological functions, the claimed polypeptides may have functions and structures that differ greatly from that of PRO1864. According to the Examiner, one of skill in the art would not be able to identify the encompassed molecules as being identical to those instantly claimed. The Examiner also asserts that the specification does not disclose any polypeptide that is 95% or 99% identical to SEQ ID NO: 14 and more highly expressed in melanoma compared to normal skin tissue.

Applicants continue to maintain that there is not substantial variation within the species which fall within the scope of the amended claims, which require at least 95% amino acid sequence identity to the disclosed sequences related to SEQ ID NO: 108 and that the pending claims are analogous to the claims discussed in Example 14 of the written description training materials. In Example 14, the written description requirement was found to be satisfied for claims relating to polypeptides having 95% homology to a particular sequence and possessing a particular catalytic activity, even though the applicant had not made any variants because there was not substantial variation within the species encompassed by the claims. Similarly, the claimed polypeptides also have very high sequence homology to the disclosed sequences and share the same expression pattern in certain tumors, or share an epitope sufficient to generate antibodies which specifically detect the polypeptide of SEQ ID NO: 14 in skin tissue samples.

In Example 14, the procedures for making variants were known in the art and the disclosure taught how to test for the claimed catalytic activity. Similarly, in the instant application, it is well known in the art how to make polypeptides with at least 95% amino acid sequence identity to the disclosed sequences. In addition, as discussed above, the specification discloses how to test to determine if the polypeptide or encoding nucleic acid is differentially expressed in melanoma, and how to make antibodies which specifically detect the polypeptide of

SEQ ID NO: 14 in skin tissue samples. Like Example 14, the genus of polypeptides that have at least 95% amino acid sequence identity to the disclosed sequences will not have substantial variation.

Additionally, the Examiner asserts that the claims encompass polypeptides where the only distinguishing characteristic is partial structural identity with SEQ ID NO: 14, such as 95% or 99% amino acid sequence identity with amino acids 21-53, 119-129 or 167-234 of SEQ ID NO: 14. The Examiner maintains that there is no functional limitation with respect to these partial structures of SEQ ID NO: 14 and that the encompassed polypeptides may have substantially different structures and biological functions. The Examiner asserts that this situation is not similar to Example 14 of the written description training materials, which is drawn to polypeptides having 95% homology to a particular full-length sequence and possessing a particular catalytic activity.

As discussed above, Applicants maintain that, in view of the high degrees of homology recited Claims 4-5 and 12-17, there is not substantial variation within the species falling within their scope. Furthermore, Applicants maintain that the claims contain functional limitations which also contribute to the lack of variability within the encompassed species. In particular, Claims 4, 5, 12 and 13 specify that the claimed polypeptides are more highly expressed in melanoma compared to normal skin tissue or are encoded by a polynucleotide that is more highly expressed in melanoma compared to normal skin tissue. Claims 14-17 specify that the claimed polypeptides or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 14 in skin tissue samples. Accordingly, Applicants maintain that, just as the written description requirement was satisfied in Example 14 of the written description training materials because there was not substantial variation within the species encompassed by the claims at issue therein, the present claims also satisfy the written description requirement in view of the lack of substantial variation in the species falling within their scope.

Furthermore, in a recent Federal Circuit decision, *In re Wallach*, 378 F.3d 1330, 1333-34 (Fed. Cir. 2004), the Court stated:

> [W]e agree with Appellants that the state of the art has developed such that the complete amino acid sequence of a protein may put one in possession of the genus

> of DNA sequences encoding it, and that one of ordinary skill in the art at the time the '129 application was filed may have therefore been in possession of the entire genus of DNA sequences that can encode the disclosed partial protein sequence, even if individual species within that genus might not have been described or rendered obvious. ... A claim to the genus of DNA molecules complementary to the RNA having the sequences encompassed by that formula, even if defined only in terms of the protein sequence that the DNA molecules encode, while containing a large number of species, is definite in scope and provides the public notice required of patent applicants.
>
> ***
>
> Moreover, we see no reason to require a patent applicant to list every possible permutation of the nucleic acid sequences that can encode a particular protein for which the amino acid sequence is disclosed, given the fact that it is, as explained above, a routine matter to convert back and forth between an amino acid sequence and the sequences of the nucleic acid molecules that can encode it. *Id.* (emphasis added).

Given the degenerate nature of the genetic code, a large polypeptide is encoded by a vast number of different nucleic acid sequences. Yet the Court did not require the Applicants in *Wallach* to actually make and individually describe all of the sequences which encode the disclosed polypeptide sequence. Because it is routine to convert between amino acid sequences to nucleic acid sequences, disclosure of a single amino acid sequence was sufficient to describe the very large genus of nucleic acids which could encode the polypeptide sequence.

The facts in *Wallach* are very similar to the instant case. Here, Applicants have disclosed SEQ ID NO: 14, and claim polypeptides which are homologous to it and have the functional limitations of being more highly expressed in melanoma compared to normal skin tissue, being encoded by a polynucleotide that is more highly expressed in melanoma compared to normal skin tissue, or being able to be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 14 in skin tissue samples. As discussed above, routine methodology may be employed to make variant polypeptides, to generate antibodies to detect the presence of a polypeptide in a sample or to detect the presence of a polynucleotide encoding a polypeptide in a sample. Such methodology is as predictable and easy as creating all of the nucleic acids which encode a particular amino acid sequence. The foregoing structure/function combinations are sufficient to describe the claimed polypeptides. The *Wallach* opinion makes

clear that there is no need to list each individual sequence within the genus, or be able to visualize their detailed chemical structure, to adequately describe the genus.

Accordingly, for the foregoing reasons, Applicants respectfully request that the rejection of Claims 4-5 and 12-17 for failing to satisfy the written description requirement be withdrawn.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

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